Yeast cell-wall products containing β-glucan plus ascorbic acid affect neonatal *Bos taurus* calf leukocytes and growth after a transport stressor[1]

S. D. Eicher,*[2] I. V. Wesley,† V. K. Sharma,† and T. R. Johnson‡

*USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN 47907; †USDA-ARS, National Animal Disease Center, Ames, IA 50010; and ‡Purdue University, West Lafayette, IN 47907

ABSTRACT: The objectives were to ascertain whether a yeast cell-wall derivative that was 1.8% β-glucan in combination with ascorbyl-2-polyphosphate could improve innate immunity and mediate transportation stress in neonatal calves, and to compare the 1.8% β-glucan yeast cell-wall derivative with a more purified yeast cell-wall derivative (70% β-glucan). Treatments were 1) an unsupplemented control (CNT); 2) 113 g of a 1.8% (approximately 2%) β-glucan derivative of yeast cell walls plus 250 mg of L-ascorbic acid phosphate (BG2); or 3) 150 mg of a purified β-glucan fraction from yeast cell walls (approximately 70% β-glucan) plus 250 mg/feeding of L-ascorbic acid phosphate (BG70). Calves (n = 39) were transported for 4 h, placed in outdoor hutches, and randomly assigned to treatments. Treatments (mixed with a milk replacer) were individually fed twice daily for 28 d. Calves were offered calf starter, free choice, throughout the study. Weekly starter intake and BW were measured, and fecal samples were collected for *Salmonella* Typhimurium and *Escherichia coli* O157:H7 PCR analysis. Blood was collected immediately before transport (d 0) and on d 3, 7, 10, 14, 21, and 28 after transport. Starter intake and DMI were less ($P < 0.05$) at d 28 for the BG2 and BG70 treatments compared with the CNT treatment. Hematocrit percentages increased ($P = 0.002$) throughout the experiment. White blood cell counts (treatment × time interaction, $P = 0.066$) were less for the calves supplemented with BG70 than for those supplemented with BG2 ($P = 0.01$) or for CNT calves ($P = 0.04$) on d 28. Granulocyte counts changed ($P = 0.04$) throughout the experiment. A trend ($P = 0.077$) for a treatment × time interaction was detected for peripheral blood mononuclear cell counts (PBMC). Counts of PBMC were greater ($P = 0.006$) for the BG2 treatment compared with the CNT treatment on d 3. Calves given the BG70 supplement had fewer PBMC than those given the BG2 supplement on d 21 ($P = 0.03$) and 28 ($P = 0.05$). Fibrinogen concentrations were affected only by time ($P = 0.002$). Time effects were detected for phagocytosis ($P = 0.005$), oxidative burst ($P < 0.001$), expression of cluster of differentiation 18 ($P = 0.001$), and increased cluster of differentiation 18 ($P = 0.006$). Phagocytosis was less ($P = 0.05$) for calves in the BG70 group than for those in the CNT group. Percentage of calves positive for *E. coli* O157:H7 was greatest ($P \leq 0.05$) for those in the BG2 group on d 7 compared with those in the other treatments. The BG2 and BG70 supplements both increased feed intake, but only the BG2 supplement increased *E. coli* shedding on d 7, and the BG2 and BG70 supplements varied in modulating immune functions, indicating differences in yeast extract function.

Key words: cattle, β-glucan, immunity, transportation, vitamin C, yeast cell wall

J. Anim. Sci. 2010. 88:1195–1203
doi:10.2527/jas.2008-1669

INTRODUCTION

Transporting dairy cattle multiple times during their lives has become a routine practice because of farm specialization for different ages of growing calves and heifers, and because sometimes environmental protection issues can limit the number of animals that can be kept on the property. The effect that early transport may have on neonatal calves has not been well established. However, age at transport (<1 to >3 wk)

[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

[2]Corresponding author: Susan.Eicher@ars.usda.gov

Received November 21, 2008.
Accepted November 12, 2009.

is negatively correlated with morbidity and mortality (Knowles, 1995; Eicher, 2001).

Dietary supplements are possible immune modulators that may assist calves during immunity development and frequent managerial stressors. Two such supplements are yeast extracts (specifically β-glucan components) and vitamin C (ascorbic acid). Beta-glucan has been shown to modulate innate immune function in several species (Pedroso, 1994; Rodriguez et al., 2003; Lowry et al., 2005). Ascorbic acid also modulates innate immunity and health measures. Neonatal dairy calves fed a diet containing ascorbic acid had less nasal and ocular discharge, which indicated better mucosal health (Eicher-Pruiett et al., 1992).

Fewer studies have examined the effects of β-glucans and ascorbic acid when administered together. Eicher et al. (2006) reported a synergistic relationship with a yeast extract with β-glucans and ascorbic acid on pig growth and feed efficiency. Modulation of fish innate immune responses occurred when β-glucan was fed with ascorbic acid (Verlhac et al., 1998).

The objectives of this study were to ascertain if a yeast cell-wall derivative that was 1.8% β-glucan in combination with ascorbyl-2-polyphosphate could improve innate immunity and mediate transportation stress in neonatal calves, and to compare the 1.8% β-glucan yeast cell-wall derivative with a more purified β-glucan (70% β-glucan) yeast cell-wall derivative.

MATERIALS AND METHODS

Animal care and use was approved by Purdue Animal Care and Use Committee.

Animals and Treatments

Calves from the Purdue Dairy Teaching and Research Center were used for the study (20 bulls and 19 heifers). Calves were transported once on Monday, when they were at least 3 d of age and less than 10 d of age (mean age = 6 d). Variables were measured 28 d after the transport. All transport occurred during moderate fall and spring climates (4.4 to 27.8°C and 50 to 73% humidity; n = 16 in fall, and n = 23 in spring). At approximately 24 d of age (±5 d), calves were dehorned. No vaccinations were given during the study.

The 39 calves were blocked by birth date and assigned randomly to 1 of 3 treatments (n = 13 calves/treatment) in a randomized complete block design. Treatments were 1) a positive control of a 1-time subcutaneous electrolyte treatment (Plasma-lyte A, Baxter Health, Deerfield, IL; containing 140 mEq/L of Na^+, 5 mEq/L of K^+, 3 mEq/L of Mg^{2+}, 98 mEq/L of Cl^-, 23 mEq/L of gluconate, and 27 mEq/L of acetate; **CNT**); 2) a yeast cell-wall derivative containing 1.8% (rounded as 2%) β-glucan (EnergyPlus, Natural Chem Group LLC, Houston, TX) plus ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ; **BG2**); or 3) β-glucan extracted from yeast cell walls (approximately 70% β-glucan; BetaRight, BioThera, Eagan, MN) plus ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ; **BG70**). Supplement amounts were 113 g/feeding of 1.8% β-glucan and 250 mg/feeding of ascorbyl-2-polyphosphate, 150 mg/feeding of 70% β-glucan (equivalent to the β-glucan component of BG2) and 250 mg/feeding of ascorbyl-2-polyphosphate, or subcutaneous electrolytes without glucose. Supplements were delivered twice daily throughout the study and electrolytes were given 1 time only. Ascorbyl-2-polyphosphate alone was not tested as a treatment in this experiment because in preliminary work (our unpublished results), yeast extract and ascorbyl-2-polyphosphate acted synergistically and the objective of this experiment was to ascertain the effects of the β-glucan component of yeast extract in the combination of supplements.

Approximately 2.5 h after the morning feeding, the calves were weighed, blood was collected by jugular venipuncture, a fecal sample was collected, and calves were loaded by walking up a ramp onto a 4.88-m aluminum trailer. Transport consisted of a 4-h journey (0800 to 1200 h). The trailer was bedded with approximately 10 cm of straw, and space was restricted to allow space (approximately 1 m^2/calf) for each calf to lie down. On return to the dairy, CNT calves were given the subcutaneous electrolytes (37°C, given equally over each shoulder), and all calves were placed in individual outdoor polyethylene hutches (2.18 × 0.97 m) with wire fencing (approximately 1.5 × 0.97 m). Supplements were fed in milk replacer after the transport, beginning with the first evening feeding. Calves were weighed weekly at approximately 2.5 h after the morning feeding. Jugular blood samples were collected in 8.5-mL acid citrate dextrose vacuum tubes (trisodium citrate = 22.0 g/L, citric acid = 8 g/L, and dextrose = 24.5 g/L) before transport on d 0 and after transport on d 3, 7, 10, 13, 21, and 28. Samples were stored on ice until processed in the laboratory.

Calves were fed 4 L of pooled colostrum within 12 h after birth and were fed twice daily for the first 2 d of life. From d 3 throughout the remainder of the experiment, calves were fed 4.45 kg/d of a 20% protein and 20% fat (all milk protein) milk replacer. Milk replacer was reconstituted at 12.5% milk solids using a wire whisk and was divided into 2 equal feedings per day throughout the 28-d experiment. At each feeding (0600 and 1530 h), supplement treatments were added to batches of bucket-fed reconstituted milk replacer by treatment. Grain-based starter (formulated to provide 21% CP) was provided free choice.

Jugular blood was collected into 10-mL heparinized tubes from calves after the first 2 feedings of colostrum, between 24 and 48 h after birth. Plasma was removed after centrifugation of blood at 1,800 × *g* at 4°C for 20 min for confirmation of adequate passive transfer of im-

munoglobulins. All calves were determined to have adequate immunoglobulin absorption by plasma protein concentration determination (passive transfer; >5.5 g of protein/dL of plasma; McVicker et al., 2002).

Sample Analysis

Differential cell counts of total white blood cells, peripheral blood mononuclear cells (**PBMC**), and granulocytes, and hematocrit percentages and fibrinogen concentrations were measured using a QBC VetAuto-Read Hematology Analyzer (IDEXX, Westbrook, MA). Phagocytosis and oxidative burst of neutrophils were determined using a modification of the whole blood assay of Böhmer et al. (1992). Whole blood (450 μL) was incubated at 37°C for 1 h before adding 50 μL of dihydrorhodamine (29 m*M*; Calbiochem, San Diego, CA) to each tube. After a 10-min incubation, 50 μL (time 0) was removed and placed into 1 mL of lysing buffer (Sigma, St. Louis, MO) for 1 min. Isotonicity was restored by adding 1 mL of 1× Hanks' Balanced Salt Solution (**HBSS**; Invitrogen Corporation, Carlsbad, CA). Fifty microliters of Pansorbin (Calbiochem), which had been incubated with propidium iodide (100 mg/mL) for 30 min and washed twice, was added to the 450 μL of blood stained with dihydrorhodamine. After incubation at 37°C for 10 min, a 50-μL sample (time 10) was removed and lysed as with the time-0 sample. All samples were washed twice and resuspended in HBSS. The time-0 cells served as the control cells for each sample. Percentages of cells expressing cell surface cluster of differentiation (**CD**)18 (adhesion molecule) were measured using flow cytometry. Whole blood (500 μL) was incubated at 23°C for 30 min using fluorescein isothiocyanate-labeled CD18 (Dako, Carpinteria, CA). After lysing and resuspending in HBSS, the percentage of cells fluorescing and the mean fluorescence were determined for 5,000 cells, using a Coulter Elite flow cytometer (Beckman Coulter Inc., Hialeah, FL) with a 488-nm air-cooled argon laser for excitation and a 525-band pass for fluorescein isothiocyanate labels and a 575-band pass for PE labels. Percentage of cells expressing CD18 above the control cells and percentage of cells exhibiting increased fluorescence of CD18 above a second gate were calculated.

Microbial Analysis

Fecal samples were kept on ice for transport to the laboratory and frozen immediately at −80°C. The samples were shipped to the National Animal Disease Center, USDA-ARS, Ames, IA, for analysis of *Salmonella* Typhimurium and *Escherichia coli* O157:H7.

Enrichment of Salmonella Typhimurium and E. coli, and Extraction of the PCR Template. Feces were weighed and 1:10 (wt/vol) of gram-negative broth:tripicase soy broth was added to a 50-mL conical centrifuge tube, vortexed, and incubated (6 to 18 h at 37°C with shaking). All centrifugations were at 25°C. Tubes were centrifuged (1,000 × *g* for 2 min), and then 50 μL of the clarified liquid was transferred to a screw-capped tube containing 950 μL of gram-negative broth:tripicase soy broth and centrifuged (2 min at 14,000 × *g*), and the supernatant was discarded. To the remaining pellet, 200 μL of InstaGene matrix (Catalog No. 732-6030, Bio-Rad Laboratories, Hercules, CA) was added, the tube was vortexed for 10 s and incubated (boiling water bath for 8 to 10 min), vortexed for 10 s, and centrifuged at 14,000 × *g* for 3 min. One-hundred microliters of supernatant was transferred to a fresh tube (avoiding the pellet) and frozen immediately at −80°C until used in PCR analysis. One microliter of supernatant was used per 10 μL of PCR reaction volume.

Detection of Salmonella Typhimurium Phagetype DT104 and E. coli O157. The presence of *Salmonella* Typhimurium and *E. coli* O157:H7 serotypes in the enrichments was detected by multiplex real-time PCR using primers and probes (Carlson et al., 1999; Sharma and Carlson, 2000). However, the *E. coli* O157:H7 primers and probe were replaced with a newer set developed recently (Sharma, 2002). Conditions for PCR were 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 3 m*M* $MgCl_2$, 0.2 m*M* deoxynucleotide 5′-triphosphate, 450 n*M* of each sipB-sipC (DT104) primer, 300 n*M* of *E. coli* primer, 100 n*M* of each fluorogenic (TaqMan) probe, and 2.5 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Cycling conditions included a 10-min, 94°C activation step followed by 40 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 45 s. On completion of the 40 cycles, the data were analyzed using Applied Biosystems Sequence Detection System software. Positive reactions were determined by examining the $R_n/\Delta R_n$ (where R is the cycle threshold) and the increase in fluorescence of individual samples.

Statistical Analysis

Data were analyzed as a randomized complete block design with repeated measures over time using a mixed model (PROC MIXED, SAS Inst. Inc., Cary, NC) with a compound symmetry covariance structure specified (Littell et al., 1996). Model statements included terms for the fixed effects of treatment and time and treatment × time interactions, including the baseline pretransport data as time 1. Block (birth date) and sex were tested as random variables. Sex was removed from the model because it was not significant. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. However, when weak trends ($P \leq 0.15$) for interactions were found, pair-wise comparisons were separated using the Fisher protected LSD in SAS. Chi-squared analysis in SAS was used to evaluate the presence of microbial populations, with α set at 0.05.

Table 1. Means (±SE) of BW and feed intake (as fed) of calves either provided a 1-time subcutaneous electrolyte injection (control; CNT) immediately after a 2-h transport or fed supplements containing 2 or 70% β-glucan extracts from yeast cell walls (as fed) plus 250 mg/feeding of ascorbyl-2-polyphosphate (BG2 or BG70, respectively) for 28 d after transport

Item[1]	*P*-value[2]	Before transport, d 0	Day after transport			
			7	14	21	28
BW, kg						
CNT	Time = 0.001	44.5 ± 1.4	45.9 ± 1.3	47.8 ± 1.3	51.4 ± 1.6	55.4 ± 1.34
BG2		43.9 ± 1.4	46.0 ± 1.3	47.4 ± 1.4	50.9 ± 1.6	55.2 ± 1.34
BG70		42.6 ± 1.6	45.0 ± 1.5	46.5 ± 1.6	49.2 ± 1.8	54.1 ± 1.34
ADG, g						
CNT	Time = 0.001	—	259 ± 79	297 ± 79	492 ± 79	492 ± 67
BG2		—	285 ± 73	203 ± 73	431 ± 78	506 ± 64
BG70		—	357 ± 82	166 ± 86	376 ± 90	531 ± 75
Calf starter intake, g/d						
CNT	Treatment × time = 0.007	—	131 ± 101	325 ± 101	476 ± 101	1,032 ± 88[a]
BG2	Time = 0.001	—	85 ± 101	133 ± 101	501 ± 101	653 ± 88[b]
BG70		—	69 ± 117	159 ± 117	524 ± 117	620 ± 101[b]
DMI,[3] g/d						
CNT	Treatment × time = 0.007	—	650 ± 103	843 ± 103	995 ± 103	1,552 ± 103[a]
BG2	Time = 0.001	—	614 ± 95	703 ± 95	1,057 ± 95	1,161 ± 82[b]
BG70		—	593 ± 108	697 ± 108	1,029 ± 108	1,036 ± 92[b]
G:F						
CNT	Time = 0.08	—	0.40 ± 0.12	0.37 ± 0.12	0.51 ± 0.12	0.35 ± 0.10
BG2		—	0.57 ± 0.12	0.32 ± 0.12	0.44 ± 0.12	0.46 ± 0.09
BG70		—	0.60 ± 0.13	0.26 ± 0.13	0.34 ± 0.13	0.64 ± 0.11

[a,b]Means within day without common superscripts differ ($P < 0.05$). Superscripts are provided when an effect was detected.

[1]Treatments: CNT = electrolyte injection (Plasma-lyte A, Baxter Health, Deerfield, IL); BG2 = yeast cell-wall derivative containing 1.8% (approximately 2%) β-glucan (EnergyPlus, Natural Chem Group LLC, Houston, TX) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ); BG70 = β-glucan (approximately 70%) extracted from yeast cell walls (BetaRight, BioThera, Eagan, MN) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products).

[2]*P*-value of significant effects or trends for main effects and interactions.

[3]DMI included milk-replacer solids and starter.

RESULTS

Growth Performance

Although BW were not different ($P > 0.10$) among treatments, intake of dry feed only on d 28 (1,032, 653, and 620 g/d for CNT, BG2, and BG70, respectively) and DMI (dry feed plus milk-replacer DM; 1,552, 1,161, and 1,036 g/d for CNT, BG2, and BG70, respectively) only on d 28 were less ($P < 0.01$) for BG2 and BG70 than for CNT (Table 1). Feed efficiency was not different among treatments ($P > 0.10$), but a trend ($P = 0.08$) for a time effect was detected such that G:F was least on d 14 and greater on d 21 and 28.

Hematology

Hematocrit and leukocyte counts are summarized in Table 2. Hematocrit percentages tended to be greater ($P = 0.09$) for the BG2 treatment than for the BG70 and CNT treatments. Hematocrit percentages increased ($P = 0.002$) throughout the 28-d experiment. White blood cell counts (treatment × time interaction, $P = 0.066$) were less for the calves given BG70 compared with those given BG2 ($P = 0.01$) and for CNT calves ($P = 0.04$) on d 28. Granulocyte counts changed ($P = 0.04$) throughout the experiment, with no differences among treatments detected ($P > 0.10$). A trend ($P = 0.077$) for a treatment × time interaction was detected for PBMC counts. Peripheral blood mononuclear cell counts were greater ($P = 0.006$) for calves treated with BG2 than for CNT calves on d 3. Calves given the BG70 supplement had fewer PBMC than calves given the BG2 supplement on d 21 ($P = 0.03$) and on d 28 ($P = 0.05$). Ratios of granulocytes to PBMC were not different ($P > 0.10$) among treatments.

Fibrinogen

Plasma fibrinogen concentration (Figure 1) was greater for calves before transport (time effect, $P = 0.002$). Fibrinogen concentrations decreased throughout the study until d 21 and 28. No differences among treatments were detected ($P > 0.10$).

Leukocyte Function

Differences among treatments in leukocyte function (Figure 2) were evident only for phagocytosis. Calves in the BG70 group had fewer ($P = 0.02$) cells positive for phagocytosis compared with calves in the CNT group and tended ($P = 0.08$) to have fewer cells positive for phagocytosis than calves in the BG2 group. Time was a significant factor for phagocytosis ($P = 0.005$), oxida-

Table 2. Means (±SE) of hematocrit and leukocyte counts of calves either provided a 1-time subcutaneous electrolyte injection (control; CNT) immediately after a 2-h transport or fed supplements containing 2 or 70% β-glucan extracts from yeast cell walls (as fed) plus 250 mg/feeding of ascorbyl-2-polyphosphate (BG2 or BG70, respectively) for 28 d after transport

Variable and treatment[1]	*P*-value[2]	Before transport, d 0	Day after transport					
			3	7	10	14	21	28
HCT, %								
CNT	Treatment = 0.09	29.4 ± 1.9	30.3 ± 2.0	29.7 ± 1.9	34.0 ± 1.9	31.7 ± 1.7	31.6 ± 1.4	34.0 ± 1.3
BG2	Time = 0.002	31.8 ± 1.8	31.9 ± 1.9	34.3 ± 1.8	33.0 ± 1.8	32.8 ± 1.6	34.4 ± 1.4	37.9 ± 1.4
BG70	Treatment × time = 0.15	28.9 ± 2.1	27.2 ± 2.2	27.6 ± 2.1	27.6 ± 2.1	29.4 ± 1.9	30.1 ± 1.7	32.6 ± 1.5
WBC, $\times 10^6$								
CNT	Treatment × time = 0.066	11.5 ± 1.0	10.1 ± 1.7	10.7 ± 1.3	11.0 ± 1.1	11.2 ± 0.9	10.5 ± 1.0	12.5 ± 1.1[a]
BG2		11.1 ± 1.0	12.4 ± 1.5	12.9 ± 1.4	10.4 ± 1.0	10.1 ± 0.8	12.2 ± 1.0	14.4 ± 1.2[a]
BG70		13.3 ± 1.1	12.6 ± 1.6	13.1 ± 1.3	10.1 ± 1.2	10.8 ± 1.0	9.1 ± 1.2	10.2 ± 1.2[b]
GRN, $\times 10^6$								
CNT	Time = 0.04	4.3 ± 0.8	4.3 ± 1.3	3.9 ± 1.0	3.8 ± 0.7	4.3 ± 0.6	3.9 ± 0.7	5.2 ± 0.9
BG2		4.2 ± 0.7	4.4 ± 1.3	5.1 ± 1.0	3.1 ± 0.7	3.4 ± 0.6	4.5 ± 0.7	5.2 ± 1.0
BG70		5.3 ± 0.9	5.0 ± 1.5	6.0 ± 1.2	3.6 ± 0.8	3.9 ± 0.6	2.6 ± 0.8	3.4 ± 1.0
PBMC, $\times 10^6$								
CNT	Treatment × time = 0.077	7.2 ± 0.7	5.8 ± 0.6[b]	6.9 ± 0.6	7.3 ± 0.6	7.0 ± 0.5	7.0 ± 0.6[ab]	7.3 ± 0.6[ab]
BG2		7.1 ± 0.7	8.2 ± 0.6[a]	7.9 ± 0.5	7.3 ± 0.5	6.9 ± 0.5	7.5 ± 0.6[a]	8.3 ± 0.5[a]
BG70		7.8 ± 0.8	7.1 ± 0.5[ab]	6.5 ± 0.6	5.6 ± 0.6	6.7 ± 0.6	5.4 ± 0.7[b]	6.4 ± 0.6[b]
GRN:PBMC								
CNT		0.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	1.0 ± 0.3
BG2		0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.3
BG70		0.9 ± 1.2	0.6 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.8 ± 0.3

[a,b]Within a day, means of a variable without common superscripts differ ($P \leq 0.05$).

[1]Variables: HCT = hematocrit; WBC = white blood cell counts; GRN = granulocytes; PBMC = peripheral blood mononuclear cells. Treatments: CNT = electrolyte injection (Plasma-lyte A, Baxter Health, Deerfield, IL); BG2 = yeast cell-wall derivative containing 1.8% β-glucan (EnergyPlus, Natural Chem Group LLC, Houston, TX) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ); BG70 = β-glucan (approximately 70%) extracted from yeast cell walls (BetaRight, BioThera, Eagan, MN) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products).

[2]*P*-value of significant effects or trends for main effects and interactions.

tive burst ($P = 0.001$), CD18 expression ($P = 0.001$), and increased fluorescent CD18 expression ($P = 0.006$). All measures of leukocyte responses increased over the 28-d experiment.

Microbial Analysis

The frequency of *Salmonella* Typhimurium DT104 detection in weekly fecal samples was not different ($P > 0.10$) among treatments (data not shown). The percentage of calves positive for *E. coli* O157:H7 was greater ($P = 0.04$) for BG2-supplemented calves than for BG70-supplemented and CNT calves on d 7 (Figure 3). There was a time effect ($P = 0.05$) of calves positive for *E. coli* O157:H7 for all treatments.

DISCUSSION

Body weight loss occurred for all treatments after transport. Although not typical, studies have shown that calves may have decreased BW gain in the second or third week of life (Eicher et al., 1994; Todd et al., 2000). This was evident in both the BG2 and BG70 treatments on d 14. Although animals from both the BG2 and BG70 treatments gained more BW in the fourth week than in the previous week, feed intake did not increase as dramatically as in the CNT treatment. The total BW of the calves by d 28 was also similar to that of the CNT calves. This observation implies that transport was not as great a stressor for which dietary supplements might be beneficial, as seen with a weaning stressor in other species. Neonatal pigs supplemented with β-glucan and ascorbic acid had improved BW gain at weaning (Eicher et al., 2006) compared with control pigs and pigs supplemented with ascorbic acid only. However, nutrient supplementation can have unexpected results when paired with stressors such as transportation. For example, feeding vitamin C alone after transport resulted in depressed keyhole limpet antibody titers of calves (Tyler and Cummins, 2003).

Relatively large morbidity and mortality rates during the weeks after transport have been observed previously (Trunkfield and Broom, 1990; Knowles, 1995). Immunological responses to a transport stressor are not evident until d 7 after the transport of neonates (Tyler and Cummins, 2003), implying that the effects of stress are delayed in young calves compared with mature cattle. Additionally, Knowles et al. (1997) noted that young transported calves do not have the same sharp increases in heart rate, plasma cortisol, and glucose that are typical of older transported animals (Eicher and Burton, 2005), which may explain some

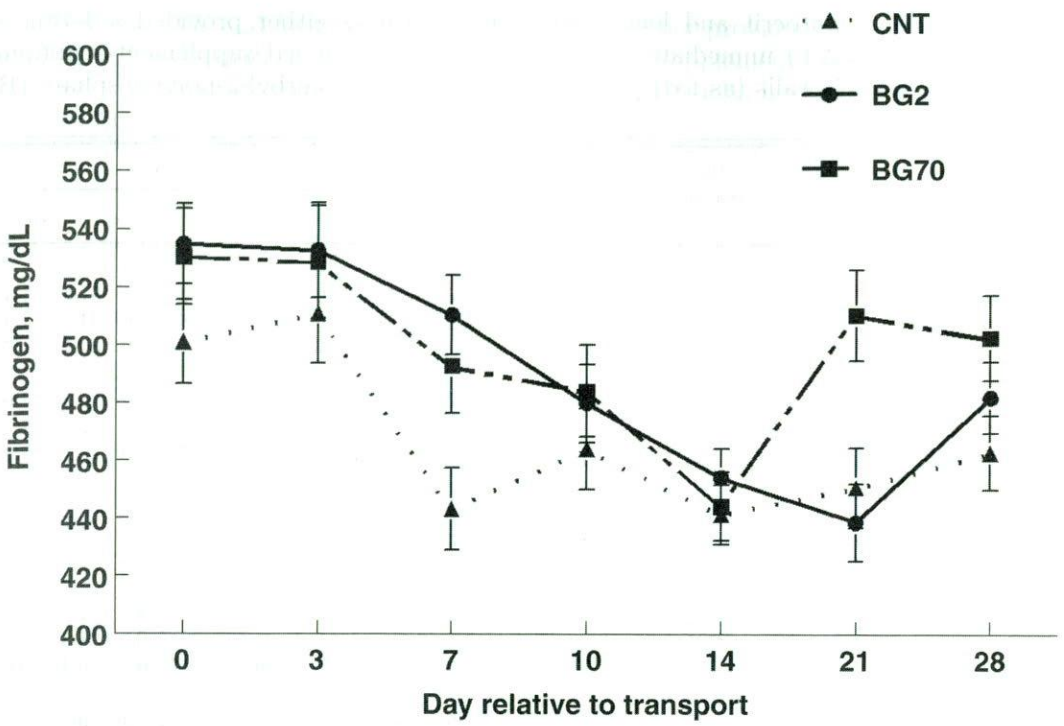

Figure 1. Plasma fibrinogen concentrations of calves given a control (CNT), 2% β-glucan plus ascorbyl-2-polyphosphate (BG2), or 70% β-glucan plus ascorbyl-2-polyphosphate (BG70) treatment. Time effect ($P = 0.002$). Data are least squares means ± SE. CNT = 1-time subcutaneous electrolyte injection (Plasma-lyte A, Baxter Health, Deerfield, IL); BG2 = 113 g/feeding of yeast cell-wall derivative containing 1.8% β-glucan (approximately 2%; EnergyPlus, Natural Chem Group LLC, Houston, TX) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ); BG70 = β-glucan (approximately 70%) extracted from yeast cell walls (BetaRight, BioThera, Eagan, MN) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products).

of the responses in the current study. However, in the present study, PBMC counts were greater for calves supplemented with BG2 and tended to be greater for calves supplemented with BG70 than for CNT calves at d 3. In contrast to the d 3 response, white blood cell counts were less for calves supplemented with the more purified β-glucan and ascorbic acid (BG70) at 28 d, and differences between the β-glucan treatments were evident in PBMC counts at d 28. This observation implies that dietary treatment takes approximately 3 wk to alter blood cell counts, but with the transport stressor, early supplementation may be beneficial by stabilizing immune responses. Additionally, we observed an overall effect of BG70, but not BG2, to suppress the phagocytosis by leukocytes to near CNT values. This implies some differential leukocyte activity that could be attributed to something other than the β-glucan factor, such as mannan and starch concentration differences in the supplements or other factors resulting from yeast growth conditions and extraction processes.

Fibrinogen is an acute phase protein that is a known indicator of stress of transported cattle (Arthington et al., 2003). Neonatal calves have increased fibrinogen concentrations after birth (Thornton et al., 1972). Calves in this study ranged from 3 to 10 d of age at transport, during which fibrinogen concentrations decrease rapidly, as reported by others (Thornton et al., 1972). Therefore, detection of treatment effects may have been obscured by the increased concentrations after birth. Fibrinogen concentrations of calves in all treatments decreased throughout the experiment to near adult concentrations.

Published data on cattle transport are derived from older, weaned calves (Steinhardt and Thielscher, 2000; Odore et al., 2004; Ishizaki et al., 2005), different from those used in the current study. However, studies of neonatal calves and transport stress have demonstrated the importance of metabolic differences in neonatal vs. older calves (Todd et al., 2000). Metabolic changes have been reported to affect the shedding of commensal and pathogenic bacteria (Bach et al., 2004). Preconditioning of mature cattle for long transports (15 h) was shown to alleviate the *E. coli* O157:H7 detected in feces (Bach et al., 2004). Our approach was to postcondition, rather than precondition, neonatal calves that were consuming primarily liquid feed. However, we noted that β-glucan increased the number of days that feces were positive for naturally occurring *E. coli* O157:H7. This observation could have 2 opposite implications. The consequences are not acceptable if we assume that feces that are positive for a pathogen are undesirable and indicative of infection and that they have potential for the spread of disease or carcass contamination at slaughter. However, if we postulate that detecting *E. coli* in the feces could indicate its clearance from the intestine and not gut colonizing, then more days of detection could

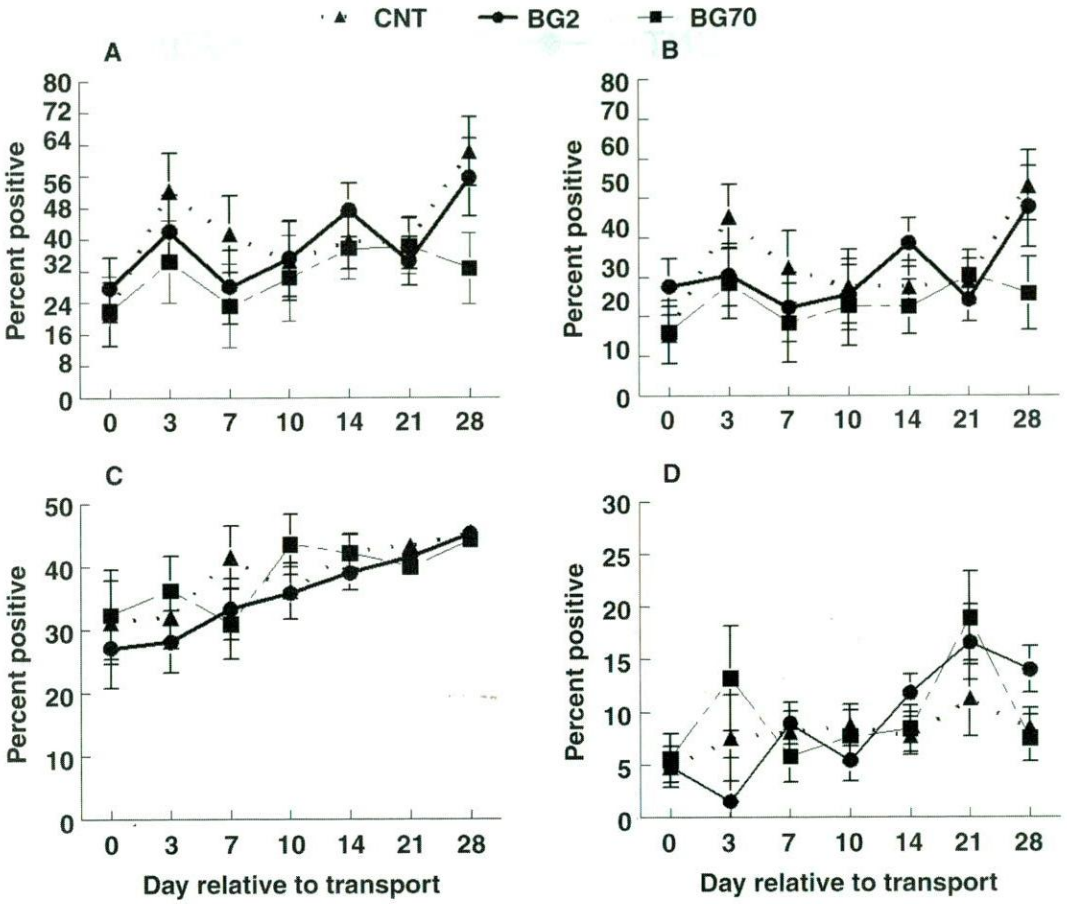

Figure 2. Percentage of cells with fluorescence from phagocytosis of unopsonized *Staphylococcus aureus* (panel A) and oxidative burst (panel B). Phagocytosis was greater ($P = 0.05$) for cells from control calves (CNT) compared with cells from calves given 70% β-glucan (BG70), and a time effect was detected ($P = 0.005$). Oxidative burst showed a trend ($P = 0.10$) for CNT calves to have more cells with fluorescence from oxidative burst compared with calves in the BG70 treatment. Percentage of cells fluorescing with fluorescein isothiocyanate-labeled cluster of differentiation (CD)18 antibody (panel C) increased over the study ($P = 0.001$). Percentage of cells that exhibited increased CD18 fluorescence (panel D) also increased over the study, peaking on d 21 ($P = 0.006$). Data are least squares means ± SE. CNT = 1-time subcutaneous electrolyte injection (Plasma-lyte A, Baxter Health, Deerfield, IL); BG2 = 113 g/feeding of yeast cell-wall derivative containing 1.8% β-glucan (approximately 2%; EnergyPlus, Natural Chem Group LLC, Houston, TX) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ); BG70 = β-glucan (approximately 70%) extracted from yeast cell walls (BetaRight, BioThera, Eagan, MN) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products).

be considered a positive indicator. Because cattle lack a Shiga toxin receptor, most *E.* coli O157:H7-infected cattle remain healthy (Pruimboom-Brees et al., 2000). However, the pathogen can live and replicate within the intestinal tract, creating a potential human health hazard when it is shed in the feces.

Therefore, we are possibly observing 2 or 3 mechanisms of supplementation with β-glucan plus ascorbic acid. First, we saw an effect on feed intake by d 28. From these data, we cannot determine if it is a physical mechanism, such as a slowing of intestinal motility, or if it is a physiological modulation of metabolism. Second, in both supplements we saw an effect of β-glucan to tend to increase the number of days that calves were positive for *E. coli* O157:H7 or the percentage of calves that were positive earlier than CNT calves. It is not known how the β-glucan interacts with a liquid diet, as opposed to a diet consisting primarily of dry feed. However, that may be an advantage of providing the β-glucan during the first 3 wk for increasing pathogen shedding rather than colonization. Third, we saw an attenuation of leukocyte function that occurred with the more purified β-glucan supplemented at lesser concentrations. There was a divergence in the response of the 2 supplements for many of the immune measures at 21 and 28 d. Therefore, there is a need to elucidate whether this is a form or concentration effect (i.e., whether the β-glucan alone, delivered at the same concentration as the mannan and β-glucan (EnergyPlus), could show the same response). Additionally, it is important to investigate whether the BG2 immune response is more protective against pathogens. Challenge studies are necessary to determine if an activated or quiescent immune system before challenge is beneficial. These data from the current study imply that these yeast cell-wall extracts plus ascorbic acid supplements may be beneficial to enhance dry feed consumption and may be beneficial to alleviate transport stress in calves, but the benefits of the 2 supplements need to be determined because they may have immunologically opposite effects.

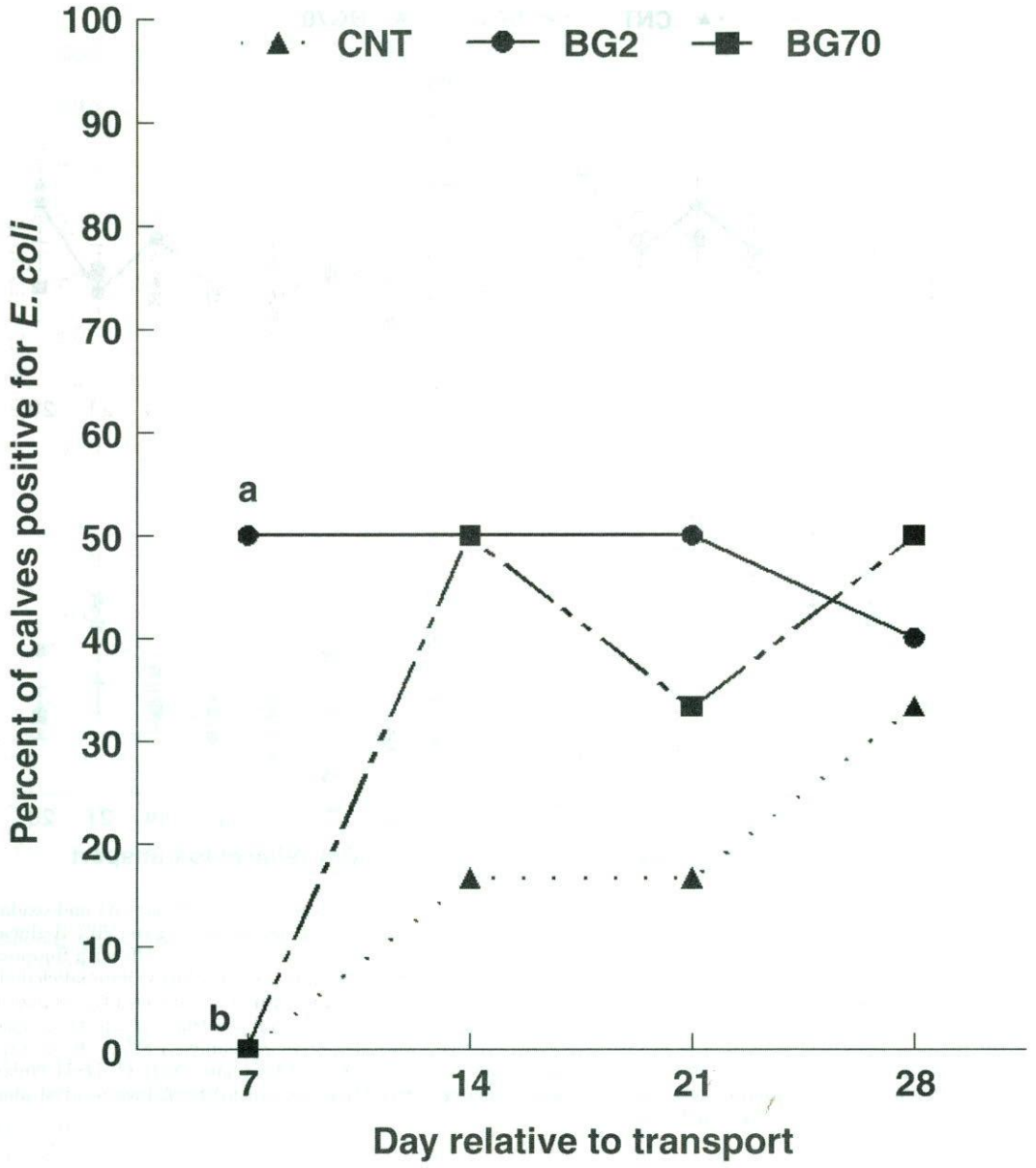

Figure 3. Percentage of days calves were positive for *Escherichia coli* O157:H7 for each treatment by week. [a,b]Means within a day without a common letter differ ($P \leq 0.05$). Data are least squares means ± SE. CNT = 1-time subcutaneous electrolyte injection (Plasma-lyte A, Baxter Health, Deerfield, IL); BG2 = 113 g/feeding of yeast cell-wall derivative containing 1.8% β-glucan (approximately 2%; EnergyPlus, Natural Chem Group LLC, Houston, TX) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products, Parsipanny, NJ); BG70 = β-glucan (approximately 70%) extracted from yeast cell walls (BetaRight, BioThera, Eagan, MN) plus 250 mg/feeding of ascorbyl-2-polyphosphate (Stay-C, DSM Nutritional Products).

LITERATURE CITED

Arthington, J. D., S. D. Eicher, W. E. Kunkle, and F. G. Martin. 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. J. Anim. Sci. 81:1120–1125.

Bach, S. J., T. A. McAllister, G. J. Mears, and K. S. Schwartzkopf-Genswein. 2004. Long-haul transport and lack of preconditioning increases fecal shedding of *Escherichia coli* and *Escherichia coli* O157:H7 by calves. J. Food Prot. 67:672–678.

Böhmer, R. H., L. S. Trinkle, and J. L. Staneck. 1992. Dose effects of LPS on neutrophils in a whole blood flow cytometric assay of phagocytosis and oxidative burst. Cytometry 13:525–531.

Carlson, S. A., L. F. Bolton, C. E. Briggs, H. S. Hurd, V. K. Sharma, P. J. Fedorka-Cray, and B. D. Jones. 1999. Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. Mol. Cell. Probes 13:213–222.

Eicher, S. D. 2001. Transportation of cattle in the dairy industry: Current research and future directions. J. Dairy Sci. 84(E-Suppl.):E19–E23.

Eicher, S. D., and J. L. Burton. 2005. Immune system: Stress effects. Pages 544–547 in Encyclopedia of Animal Science. W. G. Pond and A. W. Bell, ed. Marcel Dekker, New York, NY.

Eicher, S. D., C. A. McKee, J. A. Carroll, and E. A. Pajor. 2006. Supplemental vitamin C and yeast cell wall β-glucan as growth enhancers in newborn pigs and as immunomodulators after an endotoxin challenge after weaning. J. Anim. Sci. 84:2352–2360.

Eicher, S. D., J. L. Morrill, F. Blecha, C. G. Chitko-McKown, N. V. Anderson, and J. J. Higgins. 1994. Leukocyte functions of young dairy calves fed milk replacers supplemented with vitamins A and E. J. Dairy Sci. 77:1399–1407.

Eicher-Pruiett, S. D., J. L. Morrill, F. Blecha, J. J. Higgins, N. V. Anderson, and P. G. Reddy. 1992. Neutrophil and lymphocyte

response to supplementation with vitamins C and E in young calves. J. Dairy Sci. 75:1635–1642.

Ishizaki, H., Y. Hanafusa, and Y. Kariya. 2005. Influence of truck-transportation on the function of broncheoalveolar lavage fluid cells in cattle. Vet. Immunol. Immunopathol. 105:67–74.

Knowles, T. G. 1995. A review of post transport mortality among younger calves. Vet. Rec. 137:406–407.

Knowles, T. G., P. D. Warris, S. N. Brown, J. E. Edwards, P. E. Watkins, and A. J. Phillips. 1997. Effects on calves less than one month old of feeding or not feeding them during road transport of up to 24 hours. Vet. Rec. 140:116–124.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Inst. Inc., Cary, NC.

Lowry, V. K., M. B. Farnell, P. J. Ferro, C. L. Swaggerty, A. Bahl, and M. H. Kogut. 2005. Purified β-glucan as an antibiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis*. Int. J. Food Microbiol. 98:309–318.

McVicker, J. K., G. C. Rouse, M. A. Fowler, B. H. Perry, B. L. Miller, and T. E. Johnson. 2002. Evaluation of a lateral-flow immunoassay for use in monitoring passive transfer of immunoglobulins in calves. Am. J. Vet. Res. 63:247–250.

Odore, R., A. D'Angelo, P. Badino, C. Bellino, S. Pagliasso, and G. Re. 2004. Road transportation affects blood hormone levels and lymphocyte glucocorticoid and β-adrenergic receptor concentrations in calves. Vet. J. 168:297–303.

Pedroso, M. 1994. Application of beta-1,3-glucan to prevent shipping fever in imported heifers. Arch. Med. Res. 25:181–184.

Pruimboom-Brees, I. M., T. W. Morgan, M. R. Ackermann, E. D. Nystrom, J. E. Samuel, N. A. Cornick, and H. W. Moon. 2000. Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. Proc. Natl. Acad. Sci. USA 97:10325–10329.

Rodriguez, A., A. Cuesta, J. Ortuño, M. A. Esteban, and J. Meseguer. 2003. Immunostimulant properties of a cell wall-modified whole *Saccharomyces cerevisiae* strain administered by diet to seabream (*Sparus aurata* L.). Vet. Immunol. Immunopathol. 96:183–192.

Sharma, V. K. 2002. Detection and quantitation of enterohemorrhagic *Escherichia coli* O157, O111, and O26 in beef and bovine feces by real-time PCR. J. Food Prot. 65:1371–1380.

Sharma, V. K., and S. A. Carlson. 2000. Simultaneous detection of *Salmonella* strains and *Escherichia coli* O157:H7 with fluorogenic PCR and single-enrichment-broth culture. Appl. Environ. Microbiol. 66:5472–5476.

Steinhardt, M., and H. H. Thielscher. 2000. Reactions of dairy calves exposed to transport stress at 60 days of age. Effects of various rearing conditions in the developmental quality of calves on physiological variables and their changes. Dtsch. Tierarztl. Wochenschr. 107:59–65.

Thornton, J. R., R. A. Willoughby, and B. J. McSherry. 1972. Studies on diarrhea in neonatal calves: The plasma proteins of normal and diarrheic calves during the first ten days of age. Can. J. Comp. Med. 36:17–25.

Todd, S. E., D. J. Mellor, K. J. Stafford, N. G. Gregory, R. A. Bruce, and R. N. Ward. 2000. Effects of food withdrawal and transport on 5- to 10-day-old calves. Res. Vet. Sci. 68:125–134.

Trunkfield, H. R., and D. M. Broom. 1990. The welfare of calves during handling and transport. Appl. Anim. Behav. Sci. 28:135–152.

Tyler, P. J., and K. A. Cummins. 2003. Effect of dietary ascorbyl-2-phosphate on immune function after transport to a feeding facility. J. Dairy Sci. 86:622–629.

Verlhac, V., A. Obach, J. Gabaudan, W. Schuep, and R. Hole. 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 8:409–424.